Patent claims

1. Method for the analysis of a sample of genetic material for detailed sequence information contained in a large set of distinct sequences of the sample (the "target sequences"), comprising the following steps:
(1) producing an amount of nucleic acid templates containing the target sequences by multiplexed amplification of the sample of genetic material,
(2) using a chip with spatially separated locations containing a photocleavable oligonucleotide probe each for each target sequence to be investigated, the probes covalently bound to the chip surface,
(3) modifying, in a single reaction vessel and by using the templates produced in step (1), all oligonucleotide probes on the chip synchronously in a template-dependent manner so that the information under investigation is transferred from the target sequences of the templates to the probes,
(4) cleaving and mass spectrometrically measuring the spatially separated probes, and
(5) extracting the detailed sequence information from the mass measurements of the probes.

2. The method according to claim 1, wherein the mass of the probes is measured in a time-of-flight mass spectrometer by ionization through laser desorption pulses.

3. The method according to claim 1, wherein the target sequences are amplified before analysis in a single-vessel reaction.

4. The method according to claim 1, wherein the immobilized probes on the solid substrate are purified from contaminations and released from the template nucleic acid by intensive and, if necessary, denaturing washing after modification.

5. The method according to claim 1, wherein the probes are released from the solid substrate by irradiation after modification and purification and thereby are made accessible for mass spectrometric analysis.

6. The method according to claim 2, wherein the photolytic cleavage of the oligonucleotide probes from the solid substrate surface occurs simultaneously with their desorption and ionization in the laser desorption pulse.

7. The method according to claim 5, wherein mass spectrometric detection is performed by MALDI-TOF.

8. The method according to claim 1, wherein the probes are immobilized on a surface suitable for MALDI-TOF spectrometry, the modification of the probes occurs at this surface, and the photolytic release occurs during the MALDI-TOF measurement.

9. The method according to claim 1, wherein the modification of the photocleavable probes occurs by a template-dependent primer elongation.

10. The method according to claim 9, wherein at least one dideoxynucleotide is inserted during the template-dependent primer elongation of the photocleavable probes.

11. The method according to claim 9, wherein the modification of the photocleavable probes occurs by a template-dependent ligation using suitable reporter oligonucleotides.

12. The method according to claim 11, wherein the template specificity of the ligation is raised additionally by the sequence of the reporter oligonucleotides.

13. The method according to claim 12, wherein insertion and deletion mutations are analyzed in particular by the additional template specificity of the reporter oligonucleotides.

14. The method according to claim 11, wherein the reporter oligonucleotides can carry an additional recognition group.

15. The method according to claim 14, wherein the recognition group consists of a mass, fluorescence, or affinity marker, or a photoactive group.

16. The method according to claim 1, wherein the modification of the photocleavable probe is performed by a template-dependent, endonucleolytic cleavage.

17. The method according to claim 16, wherein the endonucleolytic cleavage is performed by restriction enzymes.

18. The method according to claim 16, wherein methylation patterns of the target sequences can be analyzed by a template-dependent restriction digest of the photocleavable probes.

19. The method according to claim 18, wherein the endonucleolytic cleavage occurs using single-strand specific nucleases.

20. The method according to claim 16, wherein single strand mismatches of hybridizations between probes and target sequences can be identified by template-dependent nuclease digests of the photocleavable probes.

21. The method according to claim 16, wherein endonucleolytic cleavage occurs using double strand-specific nucleases.

22. The method according to claim 21, wherein the double strand-specific nuclease is RNase H.

23. The method according to claim 1, wherein the photocleavable oligonucleotide probes can contain at least one ribonucleotide.

24. The method according to claim 21, wherein the ribonucleotides of the photocleavable probes can only be template-dependently digested when there is perfect base pairing, leading to detection of the mismatch in the photocleavable probes.

25. The method according to claim 9, wherein the hybridization of the target sequences to the photocleavable oligonucleotide probes and their template-dependent modification can be performed cyclicly a number of times.

26. The method according to claim 25, wherein the enzymes utilized are heat stable and the reaction mixture can be repeatedly warmed directly on the chip.

27. The method according to claim 1, wherein the chip carries, on its surface, 10 to 100,000 spatially separated, photocleavable oligonucleotide probes.

28. The method according to claim 1, wherein the photocleavage site consists of an o-nitrobenzyl residue.

29. The method according to claim 27, wherein the photocleavable oligonucleotide probe is connected additionally to the surface via a spacer in such a way that the enzymatic modification of the probes is facilitated.

30. The method according to claim 27, wherein the probes are immobilized in an array format on the surface of the chip as photocleavable oligonucleotide conjugates.

31. The method according to claim 30, wherein the photocleavable oligonucleotide conjugates carry an additional functional group for immobilization.

32. The method according to claim 31, wherein the additional functional group consists of an amino, sulfhydryl, carboxyl group, biotin, anthracene or a diene.

33. The method according to claim 27, wherein the photocleavable oligonucleotide probe is synthesized directly on the surface.

34. The method according to claim 33, wherein initially the photocleavable sites are synthesized in unison, and then if necessary the spacers are synthesized.

35. Nucleic acid chips with photocleavable oligonucleotide probes according to claim 1.